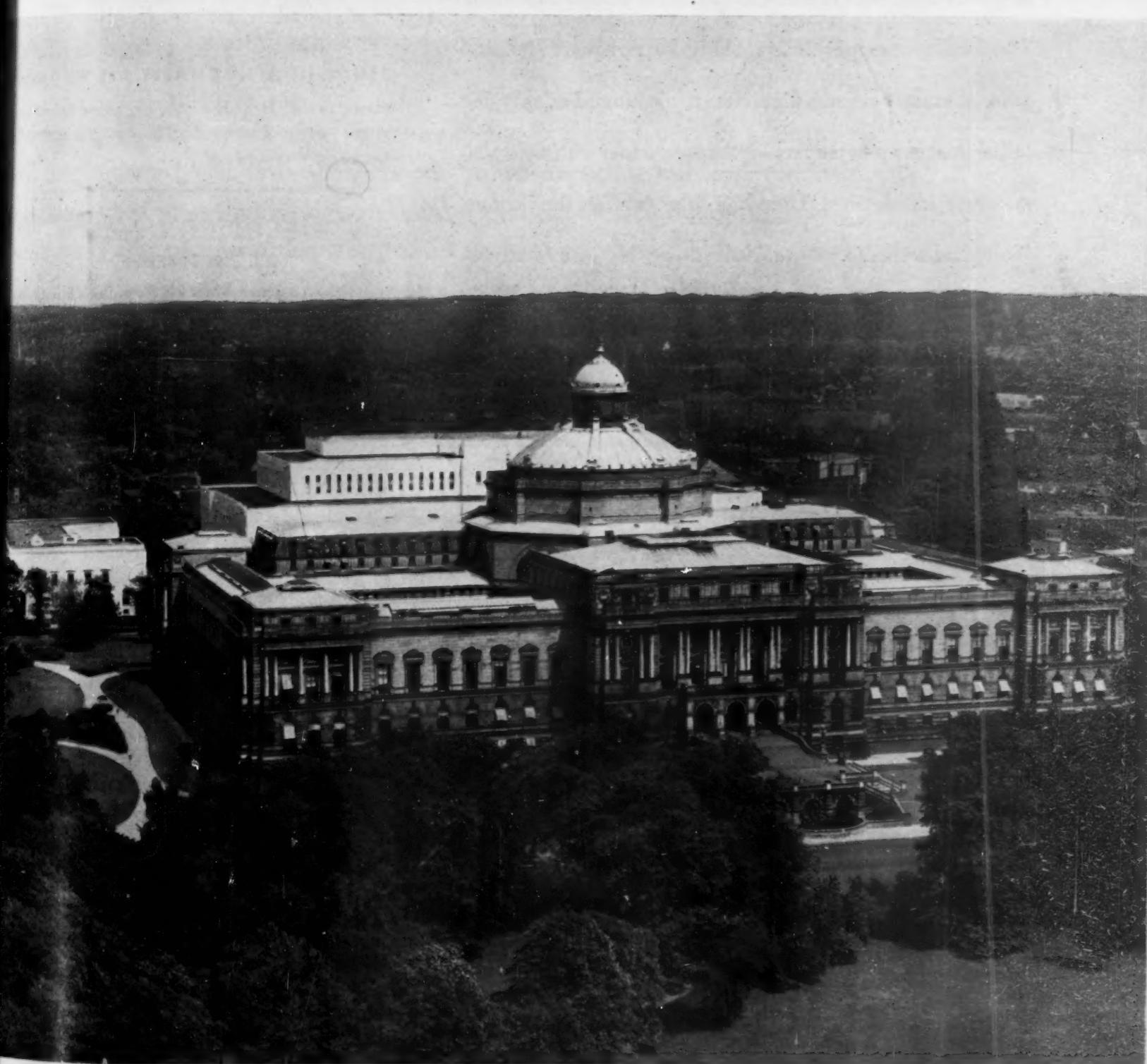


VOL. 108 • NO. 2797 • PAGES 123-146

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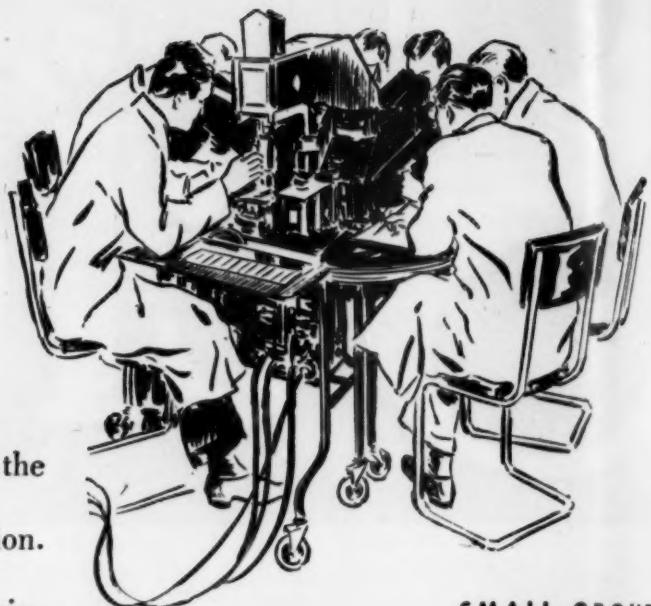
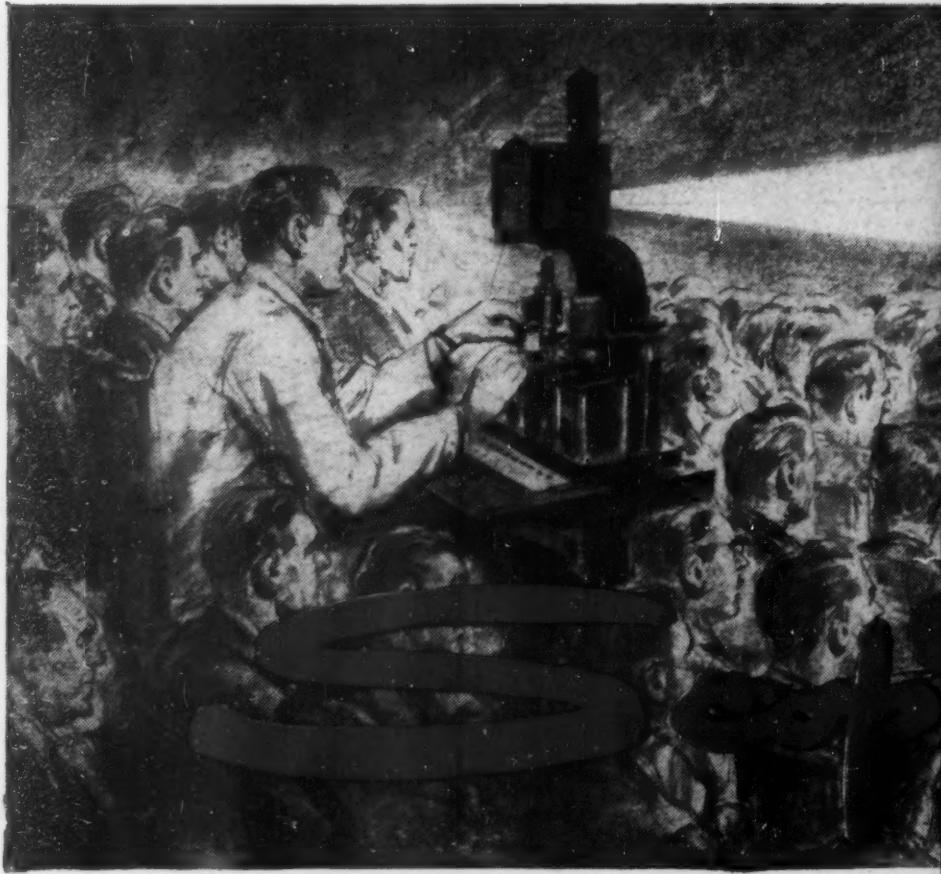
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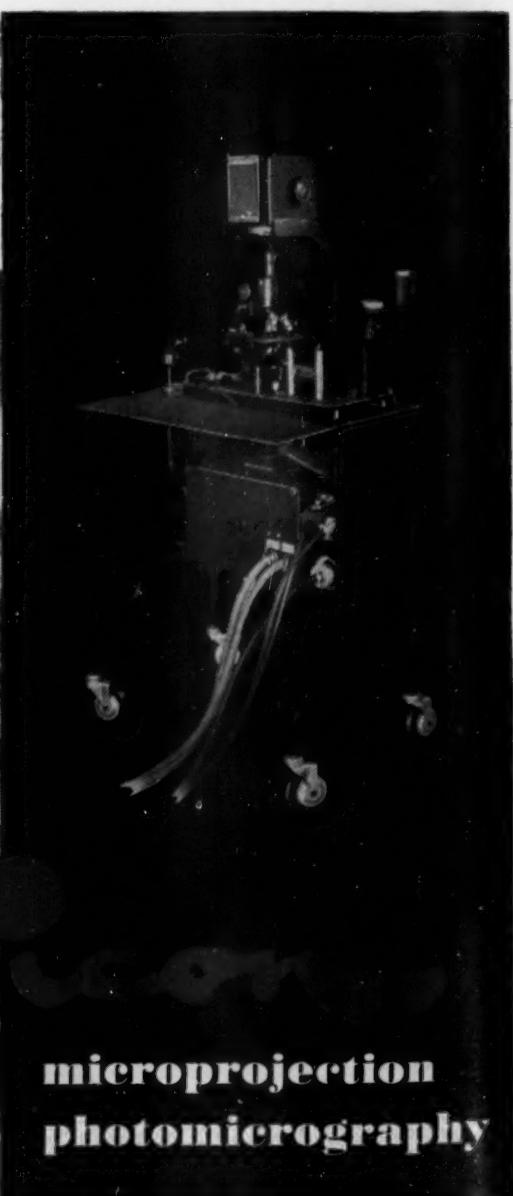


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Vol. 108 No. 2797 Friday, August 6, 1948

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Science, a weekly journal, is published each Friday by the American Association for the Advancement of Science at The Business Press, Incorporated, N. Queen St. and McGovern Ave., Lancaster, Pa. Founded in 1880, it has been since 1900 the official publication of the AAAS. Editorial and Advertising Offices, 1515 Massachusetts Avenue, N.W., Washington 5, D.C. Telephone, EXecutive 6060 or 6061. Cable address, SCIMAG, Washington, D.C. Entered as second-class matter at the Post Office at Lancaster, Pa., January 13, 1948, under the Act of March 3, 1879. Acceptance for mailing at the special rate postage provided for in the Act of February 28, 1925, embodied in paragraph 4, Sec. 538, P. L. and R., authorized January 13, 1948.

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Ecological Applications of Lansing's Physiological Work on Longevity in *Rotatoria*

W. T. Edmondson

The Biological Laboratories, Harvard University

IT IS BECOMING REALIZED WITH increasing force that studies of productivity must deal not alone with the size of populations at various times, but also with the rates at which they increase and decline as an expression of the shifting balance between reproduction and death (4, 7). One of the basic needs in such studies is information about the factors which in nature are responsible for shifting this balance. Moreover, the observed distribution of organisms in lakes of various types is in part a problem of the survival of populations as determined by these factors. Attention is here directed to work by Lansing on problems of aging in rotifers because some of the data can be used in interpreting observations on natural populations. A study of extensive data on the factors correlated with the distribution of sessile rotifers in natural waters has been published (6), and a similar set of data on free-swimming species collected by Mr. F. J. Myers in Pennsylvania is under study by the present author for future publication. Some of the latter data are presented below in preliminary form. Carlin (3) has published data on seasonal cycles of many species and fluctuations in chemical and physical factors for 6 successive years in several locations in Sweden. The usefulness of life table techniques in discussing natural mortality has been demonstrated recently by Deevey (5). In the following pages an attempt is made to show where these diverse sets of data fit together and what problems are in greatest need of investigation.

Lansing worked variously with *Euchlanis incisa* Carlin (= *triquetra* Hudson and Gosse), *Rotaria rotatoria* (Pallas) (= *Rotifer vulgaris* Schrank), *Philodina citrina* Ehrenberg, and *Proales* sp. The results which are applicable to ecological data are summarized first, and data on the natural occurrence of the species discussed subsequently. One of the general phenomena of aging in plants and animals is the gradual accumulation of calcium. Lansing (11) has confirmed this for a number of organisms, including *Euchlanis incisa*.

The effect of varying the culture medium on longevity was studied (12):

Hydrogen ion. Increasing the pH above 7.0 without changing the salt concentration of the medium in-

creased the length of the period of senility (*Euchlanis incisa*) somewhat without making a large change in mean life span, since only a few individuals survived beyond the period of fecundity. There was, however, a very large and significant increase in fecundity as the pH was increased from 6.0 to 9.6. This seems correlated with the distribution of the species in nature, described below.

Total salt concentration. The total concentration of salts in the standard culture medium was 0.04% (400 mg/liter). Survival and fecundity of *Rotaria rotatoria* and *Proales* sp. were studied in this and in the same medium diluted to 0.02%, both buffered at pH 9.4. Concentrations above 0.04% were markedly inhibitory to reproduction and survival of both species. The survival of *Proales* was slightly lower in 0.02%, but the rate of reproduction was much higher. Survival of *Rotaria* was considerably longer in 0.02%, while the rate of reproduction was about the same in both media.

Salt balance. Salt solutions (0.04%) were prepared with high, intermediate, and low calcium concentrations (about 97, 65, and 32 mg/liter) and correspondingly low, intermediate, and high potassium concentrations. Survival was significantly longer in low calcium than in the other two, where it was about equal. The rate of egg production was about the same in all three, but the total number of offspring per female must have been more in the low calcium. Parallel experiments holding potassium constant were not performed.

As a further test of the effect of calcium, the calcium was removed by treating with sodium citrate. The effects were spectacular. Immersion in citrate for a few minutes every day or so increased the mean life span of *Proales* sp. from 5.8 days in the control to 8.3 days.

Lansing's work on *Philodina citrina* and *Euchlanis incisa* (13) is in agreement with earlier work by others in showing that the mean life span is shorter for offspring born later in the mother's life than those born earlier. Hence, *orthoclones* of various ages were established and the mean life span determined. For example, eggs laid on the 8th day of a mother's life are reared, the eggs laid by these offspring on their

8th day of life are reared, etc. This is an *8-day orthoclone*, and the successive generations are designated as 8F₁, 8F₂, 8F₃, etc. Two significant facts emerged:

(1) The mean life span of animals of older orthoclones became shorter each generation until it reached zero or until no eggs were laid and the line died out.

(2) The older the orthoclone, the shorter is the number of generations until extinction. The effect is reversible, as shown by establishing young orthoclones from old, nearly played-out lines. In such cases, the parental life span is quickly regained or exceeded. The oldest orthoclone which might maintain its life span is called the isoclone. In practice, the orthoclones as seen in the laboratory have either decreasing life spans (geriaclones) or increasing life spans (pediaclones) from one generation to the next. All of these lines are, of course, parthenogenetic.

The calcium accumulation may be merely symptomatic of the progressive change in some system which results in the binding of calcium in the tissues (14). Any explanation must account for the cumulative and reversible features as well as the apparent transmission through the egg cytoplasm, and the effectiveness of calcium in the culture medium.

G. Evelyn Hutchinson has suggested (*in litt.*) that some of the well-known cyclomorphotic phenomena observed in rotifers may be correlated with aging phenomena. Thus, the changing proportions of long-spined and short-spined or spineless animals during the season or between mictic generations are possibly the result of a change of the age of the theoretical isoclone brought about by changing temperature and chemical or biological conditions. Behrens (1) suggested that the length of spines in natural popula-

TABLE 1
NUMBER OF OCCURRENCES OF SELECTED SPECIES OF ROTATORIA IN A SERIES OF LAKES OF VARIOUS PH

pH class	3.8-4.5	4.6-5.3	5.4-6.1	6.2-6.9	7.0-7.7	7.8-8.1	8.2-8.9
Species:							
<i>Asplanchnopus dahlgreni</i>		1	0	3			
<i>Lindia annecta</i>	1	1	1	2			
<i>Floscularia pedunculata*</i>	1	2	2	4			
<i>Pseudocistes rotifer*</i>	1	1	0	6			
<i>Proales fallaciosa</i>	1	4	0	2	2†		
<i>Proales sordida</i>	1	5	3	5	3	3	
<i>Keratella cochlearis</i>			1	2	3	3	
<i>Sinantherina socialis*</i>			1‡	1	6	2	3
<i>Brachionus quadridentatus</i>				2	1	2	
<i>Brachionus angularis</i>					1	2	
<i>Asplanchnopus multiceps</i>				3	1		

* Sessile species from the data of Edmondson (6); others are from unpublished data on Pennsylvania lakes.

† pH 7.0 and 7.1.

‡ Very few specimens, small colonies.

In *Philodina citrina*, which has a life span of about a month, 6-day and older orthoclones eventually die out, the 6-day line lasting for 17 generations and the 16- and 17-day lines lasting for only 3 generations under the particular experimental conditions. It is doubtless significant that the age at which the cumulative effect begins to appear is that at which growth stops (15). It is known that at cessation of growth calcium concentration in the tissues begins to increase. Possibly eggs are laid with an increasingly greater accumulation of calcium as the mother ages, so that the younger eggs are off to a better start. Other mechanisms are, of course, possible and perhaps more likely.

tions of *Keratella quadrata* is to some extent correlated with the calcium content of the water. In some investigated cases, food has seemed to be the chief factor governing cyclomorphosis. It is desirable to have a laboratory analysis made with this possibility in mind. The isoclone technique should be very useful here, and much of the conflict may be resolved by controlling factors now known to be important in aging. It is not yet known if turbulence affects rotifers in the way shown for *Daphnia* by Brooks (2), but the greater difficulty in culturing planktonic species, relative to littoral species, suggests dependence on some such obscure physical factor.

The occurrence of particular rotifer species in lakes is in many cases strikingly correlated with chemical features of the water. The nature of the correlation can be expressed conveniently in two ways—the frequency of occurrence in various classes of lakes can be tabulated to show the range and apparent optimum (Table 1), or the median concentrations of various substances can be determined and the number of occurrences above and below the median tabulated to expose any tendency to occur in particularly concentrated or dilute waters (6, Tables 2-6). The logarithmic nature of the pH scale introduces a certain complexity into such comparisons.

Harring and Myers (8) state without presenting data that hydrogen ion is the effective factor in limiting distribution and others (e.g. calcium carbonate) are not involved. This conclusion is based largely on the very sharp division which can be made between the acid and the alkaline fauna; many species have pH 7.0 as the upper or lower limit of their range of distribution and are accordingly referred to as acid or alkaline water species (Table 1). There are transversional species which occur in both acid and alkaline waters, but many of these tend to extend further into the acid than into the alkaline range. More recent work by the writer and others generally substantiates these facts, but suggests that distribution is limited by factors other than hydrogen ion (6 and unpublished; 17). In lakes, there is a high correlation between pH, calcium, and bicarbonate (9, 10), which makes the resolution of separate effects difficult in field data. Also, the generally lower productivity of acid waters limits the size of the animal populations for reasons other than chemical tolerance.

Fortunately, some of Lansing's laboratory data, including the apparently general effect of calcium, can be related to existing data on natural populations. The effect of calcium on mortality may well explain why the fauna of soft waters is so much richer in species than that of hard waters. For instance, Myers (16) reported that the fauna of Mt. Desert Island included 93 species limited to acid water, 39 to alkaline water, and 145 transversional species which occurred in both. There were 13 species which occurred in salt water. Most of the waters investigated were acid. It is very desirable to have more experimental information based on culture media in the range of salt concentration of natural waters using species of varied characteristics. Little can be said about *Philodina* or *Rotaria*, since almost nothing is known of bdelloid ecology. The author's impression is that, in nature, *Rotaria rotatoria* inhabits softer waters than any of the species of *Proales* that Lansing is likely to have had, and this agrees well with the laboratory findings, although more data are needed.

The direct effects of calcium on individual longevity are not the only ones which may be expected to affect the survival of a species in a lake, since all eggs are not equivalent. Those laid by older parents will be less effective in a survival sense than earlier eggs. A slight change in calcium concentration may be expected to have an effect on survival of the species out of proportion to its immediate effect on the individuals simply by shifting the age of the isoclone, which in turn will affect an increasing number of individuals in subsequent generations. Thus, a given rate of egg laying may be more or less effective in survival. This effect is probably amenable to a fairly simple mathematical analysis which would enable one to evaluate quantitatively the shift in terms of effective survival.

The most complete data on natural occurrence are those which concern *Euchlanis incisa*. In agreement

TABLE 2
OCCURRENCE OF *Euchlanis incisa* IN A SERIES OF LAKES
IN PENNSYLVANIA*

	No. of lakes		Maximum abundance	Temperature at maximum abundance (°C)
	Without	With		
<i>pH range</i>				
3.8-4.5	1	0
4.6-5.3	6	0
5.4-6.1	4	1	2	24
6.2-6.9	4	3	2	23
7.0-7.7	3	2	3	21
7.8-8.1	3	2	4	22
<i>Calcium range (mg/liter)</i>				
1-2	2	1	1	24
3-4	1	2	2	23
5-6	0	0
7-8	1	1	2	18
9-10	0	1	4	22
11-12	0	1	3	21
35-36	1	0

* Most of the lakes were sampled three times in one summer. Abundance in each collection was rated on a qualitative scale from 1 to 5.

with laboratory data, this species occurs in small numbers down to pH 6.0 in nature, but it is widespread and becomes abundant only in alkaline ponds (Table 2). It is thus classed as a transversional species. The species occurred all through the range observed in the lakes sampled, except that it was not found in the one lake with an unusually high calcium concentration (35.8 mg/liter). This lake contained considerably fewer species than the softer ones. The largest populations were formed at concentrations of 11.7 and 8.8 mg/liter, at the upper end of the range of occurrence. These lakes may be compared with 358 in Wisconsin, investigated by Juday, Birge, and Meloche

(10), where the range was 0.13–18.8 mg/liter. On the basis of calcium alone, *Euchlanis incisa* would be able to form large populations in about 60% of these lakes. It would be interesting to know the characteristics of fecundity and mortality of this species in culture in response to variations in calcium. It is desirable to know if "alkaline" species have a higher optimal calcium concentration than others, or whether they are responding chiefly to pH within the limits of calcium found in nature. Data are needed on fecundity and survival at various concentrations of hydrogen and other ions.

Other natural phenomena may now be examined from the present viewpoint. It is highly desirable to find out whether periodic changes of any sort that happen in natural environments have the same effect as treatment with citrate. In small, moderately mineralized ponds there may be very great diurnal fluctuations in chemistry which at one time would have been thought of as deleterious, but which may actually turn out to make the maintenance of certain species possible. Such effects should be sought.

More information on the effect of temperature is badly needed, for it seems to govern the general seasonal cycles of many species. Carlin's very extensive data include measurements of a number of chemical and physical factors as well as counts of many organisms that may serve as food for some rotifers. That chemical factors modify the effect of temperature is to be expected and may account for the different seasonal occurrences of species in different kinds of lakes (7, 18). Indeed, there is reason to believe that interaction of temperature and salinity affects the distribution of several species which tolerate wide variation in salinity from fresh water to coastal salt water. *Notholca acuminata*, for example, is found in fresh water only in the late winter and early spring, when the water is cold, but it occurs all summer in coastal bays and ponds, where the water becomes quite warm. Data are fragmentary, and this species should certainly be investigated in the laboratory. It may be expected that survival at high temperatures will be enhanced by increasing the salt content of the medium.

In summary, it may be said that the problems of the distribution of species and the productivity of populations can profitably be thought of as problems of survival and fecundity, and this throws attention

onto the physiological effects of environmental variables on the rates of reproduction, growth, and death.

The physiological mechanisms involved in the observed limitations can be determined only by properly conducted laboratory experiments, but such a laboratory study must be preceded by extensive studies of natural populations, such as those cited above, in order to find out which species are quantitatively important and which are most sensitive to environmental variations; these will be the most profitable to study. Little information of interest to ecologists will be revealed by studies of species which are rare in natural habitats or which are not limited to a more or less restricted part of the range of variation found in natural waters.

The rotifers are exceptionally useful for many kinds of physiological work, and it is likely that they will be used increasingly. The writer would like to take this opportunity to urge anyone who is contemplating a physiological study of rotifers or any other small aquatic organisms to use the above considerations in choosing his material. A little care in selecting the organisms will make the results doubly effective; it will furnish data of value to ecologists, and, further, the investigator himself will benefit by a suggestive background of observations of the behavior of the species in nature.

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Obituary

Reid Hunt 1870-1948

Reid Hunt, emeritus professor of pharmacology at the Harvard Medical School, died on March 7, 1948, after a long illness.

Dr. Hunt was born in Martinsville, Ohio, and received his early education there and at Wilmington College and Ohio University. He received his baccalaureate at Johns Hopkins, where he served as student assistant to Newell Martin. From his training with Martin he acquired a broad biological outlook on the problems of the physiological sciences which strongly influenced all his later work. After a year in Europe under Binz and Nussbaum he returned to Hopkins and received his doctorate in 1896, simultaneously acquiring his M.D. from the University of Maryland Medical School. From 1896 to 1898 he served at Columbia University Medical School as tutor in physiology. The next two years were spent in Egypt, studying the embryology of *Polipterus*. This work took him to the Upper Nile, where Kitchener was operating; indeed, he met Major Marchand at Fashoda. This incident is characteristic, since Dr. Hunt's acquaintance with important personages outside the field of science was phenomenal, despite his shyness and reticence.

On his return from Egypt he began his work in pharmacology on joining the department at Hopkins under Abel. His first work was concerned with adrenal gland extracts and with the pharmacology of epinephrine. His next paper, on the toxicity of methyl alcohol, is a classic and proved to be of great importance during the Prohibition Period, as did his *Studies in experimental alcoholism*, published in 1907.

In 1902 he went to Frankfurt to study with Ehrlich and remained there for two years. It was here that his interest in the detoxifying action of various agents was awakened and his interest in the fundamental problem of pharmacology—the relation of chemical constitution to physiologic action—firmly fixed. His preoccupation with these two problems continued throughout his life and found expression in his work on the cholines, begun not long after his return from Europe in 1904 to assume the post of chief of the

Pharmacological Division of the Hygienic Laboratory, U. S. Public Health Service. In the course of this work he discovered the amazing activity of acetyl choline, and his studies made possible the rapid identification of this substance as the active principle of Loewi's "Vagusstoff"; thus, his work enabled the humoral theory of nerve impulse transmission to secure a firm chemical background almost as soon as it was enunciated. From 1907 to 1909 he studied the relation of the iodine content of the thyroid gland and its preparations to their physiological activity and demonstrated that the activity was proportional to the iodine content. In the course of these studies he was able to demonstrate for the first time the presence of the thyroid hormone in human blood.

In 1913 he left the Hygienic Laboratory to assume the chair of pharmacology at the Harvard Medical School. Here he completed his work on the cholines and contributed a further study of quaternary ammonium compounds.

His many general activities included chairmanship of the Council on Pharmacy and Chemistry of the American Medical Association, the presidency of the Pharmacopeial Convention, and the chairmanship of the Northeastern Section of the American Chemical Society. He was the first secretary and the third president of the American Society for Pharmacology and Experimental Therapeutics and also chairman of the section on Pharmacology and Therapeutics of the American Medical Association. Until his retirement in 1935 he was consultant for the Chemical Warfare Service, U. S. Army, the Massachusetts State Board of Health, and the Hygienic Laboratory. He was also a member of the drug standardization Committee of the League of Nations. His membership in scientific bodies was extensive.

In his personal contacts he possessed great charm and was at his best in conversation with small groups. He brought to the physiological sciences a broad background of biology and chemistry and an encyclopedic knowledge of the literature. His memory will be a stimulating one for American pharmacology.

G. PHILIP GRABFIELD

Milton, Massachusetts

American Association for the Advancement of Science

The Centennial Celebration-Washington, D. C.

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Since 1870 the Library has been the sole depository for books copyrighted in this country, and its collections in American history and politics, bibliography and library science, publications of learned societies, public documents, American and foreign newspapers and maps and atlases are unequalled. Its collection of books on aeronautics is the largest in the world and its collections in technology and the sciences are outstanding. It possesses the largest collection of Chinese books outside of China and Japan and the largest collection of Russian books outside the Soviet Union.

The Manuscripts Division is the principal repository of nonarchival source material relating to American history, including, among other collections, the papers of most of the Presidents from George Washington to Calvin Coolidge. The Library is the custodian of the originals of the Declaration of Independence, the Articles of Confederation, and the Constitution of the United States.

A principal service rendered by the Library consists in making the results of its cataloguing, classification, and subject indexing of books available to other libraries through the medium of its printed catalog cards. A stock of 190,000,000 printed cards for some 2,000,000 titles is maintained by the Card Division, from which about 8,250 libraries, firms, and individuals are now purchasing copies at an annual revenue to the Government of about \$634,000. Copyright fees collected on 230,215 registrations made in fiscal 1947 totaled \$442,626, which the Library also turned over to the Treasury of the United States. The Library of Congress Trust Fund Board, established in 1925, administers gifts in the nature of endowments which now total \$2,150,000 and the income from which since its establishment has been about \$1,400,000. Since 1925 the Library has also received about \$2,900,000 in other gifts for immediate disbursement.

The National Union Catalog of books in American libraries, which is maintained as a division of the Library, contains over 14,000,000 cards for important holdings of over 700 American libraries and is ex-

tensively used for bibliographic research and inter-library loan work. At present, a Science and Technology Project, established in June 1947, is conducting research in bibliographic and allied problems on behalf of the Navy Department. Among its activities are the bringing together in one place of the many thousands of technical and scientific reports which are resulting from the Government's \$600,000,000 research and development program, and the indexing and abstracting of these reports for the use of naval contractors.

The Librarian of Congress is Luther H. Evans.

Those who plan to attend the Centennial Celebration will find much to interest them in both the library and its beautiful annex.

Symposium on the Interaction of Matter and Radiation

This symposium, to be held at 10:00 A.M. on September 15, will deal with the two divisions of elementary particle physics in which the greatest advances have been made in the last two years: (1) how an electron interacts with the electric and magnetic forces which surround it and (2) what the physical properties of the mesons, newest among the elementary particles, are.

Both are steps toward a solution of the central unsolved problem of elementary particle physics, the interrelations between 5 elementary particles (electrons, protons, neutrons, heavy mesons—mass 300, and light mesons—mass 200) and the 4 modes of transmission of energy between elementary particles (electromagnetic radiation, specific nuclear forces, neutrinos, and gravitational forces).

In contrast to the nuclear chain reaction of uranium, an application of the science of the rearrangement of nuclear particles, elementary particle physics is concerned with the transformations which produce one of these particles from another and with the forces between these particles.

The first paper on the program, "New Properties of the Electron," is by Willis Lamb, professor of physics at Columbia University. Dr. Lamb is a theoretical physicist whose wartime theoretic and experimental researches in the field of high-frequency radar devices gave him inspiration at the end of the war to apply microwave techniques to measurement of finer details of the energy levels of the hydrogen atom, not easily determined by other methods.

Dr. Lamb and others obtained remarkable results with this new technique of atomic exploration. The hydrogen atom is particularly suitable for study because its energy levels are determined by the interaction between only two particles, the proton and the

electron. But between these particles there acts not only an electrical attraction due to their equal and opposite electric charges but also magnetic forces due to the intrinsic magnetic moment associated with each particle.

The experiments to be reviewed by Dr. Lamb show that the intrinsic magnetic moment of the electron differs by a fraction of a per cent from the value which would be expected if this particle obeyed earlier simple theory.

The deviation is associated with an interaction between an electron and the electromagnetic field produced by that electron itself. In addition, Dr. Lamb has found that an effect of the same kind alters the position of all those energy levels of the hydrogen atom which describe orbits where the electron comes close to the nucleus. Although the newly observed change in energy levels might be supposed to be evidence for a new kind of interaction between the electron and the nucleus, Dr. Lamb shows that the source of the effect is again the interaction of the electron with its own electromagnetic field—an interaction which is particularly strong when this particle experiences high acceleration, as it does when near a nucleus.

Cesare M. G. Lattes, 24-year-old Brazilian scientist who already has remarkable discoveries to his credit from a previous two-year stay at Bristol, England, is, together with Eugene Gardner, of the University of California Radiation Laboratory, responsible for the striking discovery, early in 1948, that mesons are produced by the Berkeley cyclotron.

Jointly with Dr. Powell and Dr. Occhialini at the University of Bristol, Dr. Lattes discovered in the cosmic radiation at mountain elevations a new kind of meson additional to the normal meson which constitutes three-fourths of the natural cosmic radiation at sea level. The normal particle has a mass approximately 200 times as great as the electron's mass (or about 1/9 the mass of the proton or hydrogen nucleus). In contrast, the new meson found by the Bristol workers weighs approximately 300 times as much as the electron. In the work in England it was also observed that this particle often undergoes a type of spontaneous disintegration into the normal light-weight meson, with the simultaneous emission of some kind of neutral radiation not yet identified.

Dr. Lattes' paper, "Observations on Slow Mesons," in addition to summarizing these observations, will review the results of recent experiments at Berkeley on the properties of the slow mesons of both light and heavy varieties produced by the cyclotron there.

John A. Wheeler, professor of physics at Princeton University, and author with Niels Bohr of the paper on the mechanism of nuclear fission which made it possible to predict that plutonium would be suitable

for atomic bombs before that element had been isolated for experimental study, will review, in his paper on "How Mesons Disappear," the theoretical considerations which have been put forward to describe the breakup of heavy mesons into light mesons and the disappearance of light mesons, sometimes by disintegration into an electron and one or more neutral radiations, and sometimes by reaction with an atomic nucleus.

Dr. Wheeler will analyze the factors which determine the energy of the electrons which come off from

the decay of 200-mass mesons. He will also show how this process is related to the alternative process in which a light-weight meson delivers up its charge to an atomic nucleus. In particular, he will show how this process is connected with the possibility of disruption or fission of this nucleus.

The chairman of the symposium will be I. I. Rabi, professor of physics at Columbia University, winner of the Nobel prize for his work on the interaction of short-wave radiations and magnetic fields with atomic and molecular systems.

NEWS and Notes

Peter J. W. Debye, winner of the 1936 Nobel Prize in chemistry for his work on the dielectric properties of matter and for electron diffraction of molecules, has been named to succeed **John Gamble Kirkwood** as Todd professor of chemistry at Cornell University. Dr. Debye first went to Cornell as a Baker lecturer in 1940 and has since served as chairman of the Department of Chemistry.

F. A. Miller, assistant professor of chemistry at the University of Illinois, has been appointed a fellow in the Department of Research in Chemical Physics at Mellon Institute. Dr. Miller is a specialist in molecular spectra and structure.

Raymond R. Edwards, a candidate for the Ph.D. degree in inorganic chemistry at Massachusetts Institute of Technology, will join the staff of the Institute of Science and Technology, University of Arkansas, in September. Mr. Edwards has been appointed research associate in nuclear chemistry and assistant professor of inorganic chemistry.

Carl T. Parsons, until recently assistant entomologist in the Connecticut Agricultural Experiment Station, has been appointed assistant professor of zoology and entomologist at the University of Vermont and State Agricultural College, Burlington.

Shien-Siu Shu, Chinese scientist Services, University of Michigan, for the academic year 1948-49.

fields of applied mathematics and fluid mechanics, has been appointed associate professor of mathematics and research associate in mechanics at Illinois Institute of Technology. Dr. Shu came to this country in 1944 as a research associate at Brown University, from which he received the Ph.D. in 1947. He spent last year at the Institute for Advanced Study, Princeton, and this summer is working at Massachusetts Institute of Technology.

John G. Bald, who has been with the Council for Scientific and Industrial Research, Canberra, Australia, since 1928, specializing in studies on plant viruses and diseases, has joined the staff of the Division of Plant Pathology, University of California, Los Angeles, as associate professor and associate plant pathologist in the Experiment Station. His research there will be concerned with diseases of bulbous ornamental plants as part of the program of research in floricultural pathology on the Los Angeles campus.

Robert S. Pogrund, formerly of the Department of Zoology, State College of Washington, Pullman, has joined the staff of the Department of Aviation Medicine, University of Southern California School of Medicine, as assistant professor of physiology.

Daniel Robert Miller, clinical psychologist, who for the past year has held a Social Science Research Council postdoctoral fellowship, has been appointed assistant professor of psychology and chief of the Clinical Services Division, Bureau of Psychological

Robert Chambers, of the Marine Biological Laboratory, Woods Hole, Massachusetts, and the Department of Biology, New York University, has been elected foreign correspondent of the Académie Nationale de Médecine, Paris.

Margaret W. Robinson, research assistant for the Research Foundation for Alcoholism in Seattle, Washington, has recently been appointed executive secretary of the Foundation.

R. J. Jessen, acting director of the Iowa State College statistical laboratory, flew to Greece last month to develop survey plans to be used in a series of studies of health, social, and economic problems in Crete. The studies are under the general direction of **L. G. Allbaugh**, of the Rockefeller Foundation, which is sponsoring the work. **J. C. Dodson** and **N. V. Strand**, of the statistical laboratory staff, who preceded Dr. Jessen to Crete, will assist in the sample surveys.

Grants and Awards

The Research Unit on Utilization of Animal Fats at the Eastern Regional Research Laboratory, USDA, has received a departmental citation for its work "which resulted in better quality synthetic rubber and substantially increased rubber production without further expansion of plant facilities and which made possible the utilization of low-grade animal fats for making improved emulsifiers needed in the manufacture of GR-S synthetic rubber." Members of the unit include **Waldo C. Ault**, **B. A. Brice**, **M. J. Copley**, **E. T. Roe**, **B. B. Schaeffer**, and **Margaret L. Swain**.

The award of the scroll for superior service and the silver medal was made by W. A. Minor, assistant to the Secretary of Agriculture, and Louis B. Howard, chief of the Bureau of Agricultural and Industrial Chemistry, to P. A. Wells, director of the Laboratory, who represented the unit.

The Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica, has received from the King Ranch, Kingsville, Texas, grants of \$20,000 for the construction and equipment of a psychroenergetic laboratory for use in studies on heat tolerance of various classes and breeds of livestock and \$13,000 for the construction of a horse unit and the purchase of breeding animals to be used in an experimental horse-breeding project. The ranch is also making available for this program 6 quarter horse mares and a stallion. For use in a breeding program devoted to tropical cattle, the ranch is providing 50 head of Santa Gertrudis cattle, a breed of beef cattle evolved on the King Ranch from the Brahman-Shorthorn cross.

The Alvarenga Prize for this year has been awarded by the College of Physicians of Philadelphia to Melvin H. Knisely, formerly of the University of Chicago (see *Science*, July 10, p. 101), in recognition of his studies on "sludged blood." This prize was established by the will of Pedro Francisco da Costa Alvarenga, of Lisbon, Portugal, an associate fellow of the College, "to be awarded annually by the College of Physicians on each anniversary of the death of the testator, July 14, 1883." The award is usually made for outstanding work, and the recipient is invited to deliver an Alvarenga Lecture before the College.

Colleges and Universities

The eastern universities have just announced the unanimous selection of new presidents. On next January 1 Detlev W. Bronk, widely known physiologist and biophysicist, who has been director of the Eldridge Reeves Johnson Foundation for Medical Physics at the University of Pennsylvania since 1929, will take over his duties as president of The Johns Hopkins Uni-

versity in Baltimore. In this post he succeeds Isaiah Bowman, famous geographer, who has headed Hopkins since 1935 and who had asked that he be allowed to retire. Dr. Bronk, who served in an advisory capacity to many government agencies and military groups during the past war, is at present chairman of the National Research Council.

The University of Pennsylvania has named to its presidency Harold E. Stassen, former Governor of Minnesota and one of the leading candidates for the Republican Presidential nomination at the recent Philadelphia convention. Mr. Stassen succeeds George W. McClelland, who, although retiring, will assume the chairmanship of the University.

Cornell University has announced that the following retiring members of its faculty have been made professors emeriti: Walter B. Carver (mathematics), Axel F. Gustafson (soil technology), Edward S. Guthrie (dairy industry), George H. Sabine (philosophy), Herbert H. Seofield (civil engineering), O. D. von Engeln (geology), Edmund L. Worthen (soil technology), Harold J. Conn (bacteriology), N. Chandler Foot (surgical pathology), and James A. Harrar (clinical obstetrics and gynecology).

A new solvent extraction tower has recently been installed at the New York University College of Engineering for use in special research work sponsored by the M. W. Kellogg Company. The studies will be concerned with operating characteristics of all types of countercurrent extraction devices with regard to flow capacities and extraction efficiencies. The installation will be sufficiently large to yield results of industrial significance.

A postdoctoral research and training program in client-centered therapy has recently been announced by the Counseling Center and the Psychology Department at the University of Chicago. This program, which is designed for a limited number of qualified persons from the fields of clinical psychology, student counseling, psychiatry, or psychosomatic medicine, will run continuously after October 1 under the direction of Carl R. Rogers,

professor of psychology. As outlined, the program offers an opportunity to participate in staff and research seminars and in other courses being offered, to study recorded cases, to learn about and participate in the various research projects in psychotherapy now being carried on and to initiate other research in this field, and, under supervision, to carry therapeutic cases and record some of the interviews for study.

The University is waiving all tuition fees for those enrolled in the program who have a Ph.D. based in part on a psychological dissertation or who have a M.D. and are interested in psychiatry or psychosomatic medicine. Other expenses must be paid by the individual, although, if accepted, he may apply for a fellowship from an agency, foundation, or a research council. A limited number of paid positions at the Counseling Center may also be available.

The announcement notes the desirability of persons spending 12 months in the program. The age limit is 40. Further details may be obtained from Dr. Rogers, who is executive secretary of the Counseling Center at the University of Chicago, Chicago 37, Illinois.

Meetings and Elections

Final plans for the **Symposium on Steroid Hormones**, to be held at the University of Wisconsin September 6 through 8, have been announced by Edgar S. Gordon, chairman of the Symposium Committee and associate professor of medicine at the Medical School of the University of Wisconsin. This symposium is a part of the Centennial Celebration of the Wisconsin State University, and the final program is exceedingly comprehensive. It includes the chemistry, metabolism, and biological effects of the steroid hormones, and the clinical applications of these substances will be discussed in detail. Scientists from many of the representative universities and medical schools throughout this country are included in this program. The University of Edinburgh is represented by G. F. Marrian, and Canada is represented by Harry Guttridge and Eleonor Venning, of McGill University.

The meetings of this symposium will be open to the general public and physicians, chemists, and scientists from other fields are invited to participate. No registration fee is to be charged.

Facilities for the accommodation of those who are going to attend the symposium are available in Elizabeth Waters Hall on the Wisconsin campus overlooking Lake Mendota. Board and room are available at \$16.25 for the three-day period. This meeting is presented under the direction of the University of Wisconsin and the National Research Council Committee on Growth, acting for the American Cancer Society. The Wisconsin Medical School, McArdle Memorial Laboratory for Cancer Research, and the Department of Biochemistry of the University have organized the program.

The Australian and New Zealand Association for the Advancement of Science will meet in Hobart, Tasmania, January 12-19, 1949.

The Institute of Food Technologists, a professional organization of food scientists with a national membership in excess of 2,200 and with 14 regional sections distributed over the United States, held its national meeting in Philadelphia June 6-10. H. C. Diehl, director and secretary of the Refrigeration Research Foundation, Berkeley, California, was elected president of the Institute.

The International Scientific Film Association, constituted last year in Paris by delegates from 22 countries who had been invited to the inaugural congress by the Scientific Film Associations of Great Britain and France, will convene for the second time October 4-11, in London. This year's congress is being convened by Great Britain's Film Association with the help of the British Film Institute, and invitations have been sent to countries throughout the world. A feature of the 8-day program will be a Festival of Scientific Films, during which many contributions from the participating countries will be shown to members of the general public.

The primary aim of the Association is "to raise the standard and to promote the use of the scientific film and related material throughout the world

in order to achieve the widest possible understanding and appreciation of scientific method and outlook, especially in relation to social progress."

Further details about the Association and the forthcoming congress may be secured from the Scientific Film Association, 34 Soho Square, London W. 1.

The Canadian Mathematical Congress, organized in 1945, will hold a second national congress and seminar in 1949 at the University of British Columbia. Membership in the Congress is open to any full-time member of the mathematical teaching staff of a Canadian university, to those who were members of the 1945 Congress or the seminar held in 1947, or to any other person sponsored by a Congress member upon application, payment of the membership fee (\$2.00 a year), and acceptance by the Executive Committee. Applications should be sent to: Secretary, Canadian Mathematical Congress, Engineering Building, McGill University, Montreal, Canada.

The Congress has announced that, beginning in January 1949, it will publish a new quarterly journal, *Canadian Journal of Mathematics*, the editor-in-chief of which will be H. S. M. Coxeter, of the University of Toronto. The price will be \$6.00 per volume of four numbers (\$3.00 for members of the Mathematical Association of America). The managing editor, G. de B. Robinson, University of Toronto, will receive subscriptions.

The National Science Teachers Association, meeting in Cleveland, Ohio, July 3-5, considered two surveys of teaching conditions, inspected the facilities for teaching science at all grade levels in the Cleveland area, and adopted strong resolutions concerning the interest of industry in science teaching, a National Science Foundation, Federal aid to education, and the training of scientists inducted for military service. Morris Meister, principal of the High School of Science, New York City, presided. Arthur O. Baker, supervisor of sciences, Cleveland Public Schools, was chairman of the local committee.

"Science Teaching in the State of New York," a study by Paul F. Brandwein, of Forest Hills High School,

and a committee, presented a clear, over-all picture of the varied conditions in the state's high schools. Recommendations were made for improving the teachers' opportunities for effective instruction, especially in the small schools.

"The Work Week of the Science Teacher" was a report on nation-wide conditions, prepared from data collected by Earl R. Glenn, of the New Jersey State Teachers College, Montclair, New Jersey, and Harold E. Wise, of the University of Nebraska, Lincoln. It described typical and also extremes of teaching loads, class size, laboratory facilities and aid, school duties, and outside work demanded by conditions of the teaching job. Many recommendations were made, based on teachers' appraisals of the favorable and unfavorable aspects of their work.

Both "Science Teaching in the State of New York" and "The Work Week of the Science Teacher" are scheduled for publication this fall.

A tour of school gardens and nature museums of Cleveland was planned by Paul R. Young, supervisor of school gardens. A program featuring the teaching of conservation and of elementary science in the grades was planned by Miss Anna Burgess, supervising principal of the Cleveland Schools.

Among the resolutions adopted by the Association was one commending the activities of the Advisory Council on Industry-Science Teaching Relations, organized at the AAAS meeting in Chicago last December. This Council is now working out plans for increased and improved supplies of educational materials, prepared by industrial experts, for effective use by science teachers. The chairman and chief consultant for this Council are Morris Meister and G. Edward Pendray, of New York City.

Another resolution recognized the injurious effects of a previous policy of inducting scientists and science students into military service without regard to their talents or training. The resolutions urged "that our national strength be conserved and our national security increased through provisions by military authorities for an effective program of training, at the university level, of existing and potential scientists and science teach-

ers who may be inducted under the military draft legislation recently passed by Congress."

Other resolutions supported "the establishment of a National Science Foundation, with special attention to scholarships and fellowships that will train the skilled personnel required in scientific research," and renewed "our recommendations for Federal appropriations that will equalize the opportunities for effective instruction in science in the high schools of our Nation, with special attention to the discovery and development of scientific talent in youth of every state."

Officers for 1948-49, elected by mail ballot of the membership, were confirmed. They are: president, Norman G. D. Jones, biology teacher in Southwest High School, St. Louis, Missouri; president-elect, Nathan A. Neal, editorial staff, Harper & Brothers, New York City; retiring president, Morris Leister; general vice-president, Ralph V. Lefler, Department of Physics, Purdue University; secretary, Hanor A. Webb, Department of Science Education, George Peabody College for Teachers, Nashville, Tennessee; treasurer, Elbert C. Weaver, Phillips Academy, Andover, Massachusetts; eastern vice-president, Walter S. Lapp, Northeast High School, Philadelphia; north central vice-president, Emil L. Massey, supervisor of sciences, Detroit; southern vice-president, Greta Oppe, Ball High School, Galveston; western vice-president, W. Bayard Buckham, Oakland High School, Oakland, California; directors-at-large, Glenn Blough, U. S. Office of Education, Washington, D. C.; Robert H. Carleton, Michigan State College, Lansing; Leo J. Fitzpatrick, Brockton High School, Brockton, Massachusetts; Dwight E. Sollberger, State Teachers College, Indiana, Pennsylvania.

The next meeting of the National Science Teachers Association, which is an affiliate of the AAAS, will be held in Washington, D. C., December 27-30, 1948. (H. A. WEBB, Secretary.)

NRC News

The Atomic Energy Commission Postdoctoral Fellowship Board in the Medical Sciences will hold its next meeting in late September. For consideration at this meeting, applica-

tions must be sent to the Board's office in the NRC before September 1. Inquiries should be addressed to the Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C.

Deaths

A Kronstein, 81, research chemist and pioneer in the development of polystyrene, died in George Washington University Hospital, Washington, D. C., on June 13.

Harry E. Burton, who retired June 30 after nearly 40 years of service at the U. S. Naval Observatory, died July 19. His position from 1929 until his retirement was principal astronomer as head of the Equatorial Division.

George L. Streeter, 75, former director of the Department of Embryology, Carnegie Institution, died in Gloversville, New York, July 27, following a heart attack. Dr. Streeter had also served as head of the Anatomy Department at the University of Michigan and as associate editor of the *American Journal of Anatomy*.

Robert O. Moody, 83, emeritus professor of anatomy at the University of California Medical School, died in Berkeley on July 28.

Establishment in Australia of a new scientific journal has recently been announced. The new journal, to be known as the *Australian Journal of Scientific Research*, will be the responsibility of the Council for Scientific and Industrial Research in collaboration with the Australian National Research Council. It will serve as a medium for the publication of research papers of outstanding merit and its pages will be open to research workers, irrespective of country or the organization to which they are attached. N. S. Noble, editor of the new journal, will be assisted by an Editorial Board consisting of W. J. Dakin, Department of Zoology, University of Sydney; E. J. Hartung, Department of Chemistry, University of Melbourne; L. H. Martin, Department of Physics, University of Melbourne; and J. G. Wood, Department of Botany, University of Adelaide. The Journal will be issued

in two series (A, Physical Sciences; B, Biological Sciences), each of which will appear quarterly and at a cost of 30/-d per year.

A Department of Scientific Research, recently created by the Government of India, became effective on June 1 of this year. The new Department, which will be in charge of the Prime Minister of India and will have S. S. Bhatnagar, director of scientific and industrial research, as secretary and principal executive officer, will take over the work of the Board of Research on Atomic Energy and the Council of Scientific and Industrial Research. According to the announcement, the Council, although attached to the new Department, will retain its unofficial character and function as before. Also included in the list of projects with which the new Department will deal are *ad hoc* scientific research in universities and research institutions, scholarships for research in applied fields, advice to government departments, and whatever other duties may eventually be transferred to it. In coordinating the scientific activities of the other Ministries it will have the assistance of a special committee of eminent scientists.

Make Plans for—

Round Table Conference on Lignin, August 12-14, Appleton, Wisconsin.

American Veterinary Medical Association, August 16-19, Palace Hotel, San Francisco, California.

International Society of Hematology, biannual meeting, August 23-26, Hotel Statler, Buffalo, New York.

New England Association of Chemistry Teachers, annual Summer Conference, August 23-28, University of Maine, Orono.

American Institute of Electrical Engineers, August 24-27, Spokane, Washington.

American Chemical Society, 114th meeting: eastern session, August 30-September 4, Washington, D. C.; midwest session, September 6-10, St. Louis, Missouri; western session, September 13-17, Portland, Oregon.

Comments and Communications

Vitamin B₁₂, A Cobalt Complex

Vitamin B₁₂ was described by the undersigned (*Science*, April 16, p. 396) as a crystalline red compound which is highly active for producing hematological responses in patients with pernicious and other anemias (R. West. *Science*, April 16, p. 398; T. D. Spies, *et al.* *S. med. J.*, 1948, **41**, 522, 523) and for the growth of *Lactobacillus lactis* (E. L. Rickes, *et al.* and M. S. Shorb. *Science*, April 16, pp. 396, 397), chicks (W. H. Ott, E. L. Rickes, and T. R. Wood. *J. biol. Chem.*, 1948, **174**, 1047), and rats (G. A. Emerson, M. E. Zanetti, and T. R. Wood. To be published.) Independent isolation of an anti-pernicious anemia factor, presumably identical with B₁₂, has also been reported (E. L. Smith. *Nature, Lond.*, 1948, **161**, 638).

Information on the chemical nature of B₁₂ is of primary interest. The following observations on B₁₂ have been made which appear useful for other chemical and biological studies.

Emission spectrographic analysis¹ of B₁₂ has shown the presence of cobalt.² Vitamin B₁₂ appears to be a cobalt coordination complex which, having 6 groups about the cobalt atom, could involve one or more organic moieties. The red color of B₁₂ appears to be at least in part associated with its cobalt-complex character.

The presence of cobalt in vitamin B₁₂ reflects significantly upon many biological studies which have shown that cobalt is an essential trace element in nutrition, and perhaps upon suggestions concerning cobalt as a trace contaminant in iron therapy of anemias (E. J. Underwood. *Proc. Soc. exp. Biol. Med.*, 1937, **36**, 296). The nutritional significance of cobalt must be re-evaluated as the biological function of B₁₂ is developed.

Cobaltous ion (1 $\mu\text{g}/\text{ml}$) was without activity for *L. lactis* as contrasted with the high potency of B₁₂ (0.000013 $\mu\text{g}/\text{ml}$, half-maximal growth).

Randolph West has tested cobalt ion in two cases of pernicious anemia with negative results.³ The average adult daily dietary intake of cobalt has been estimated at 100 μg (B. Ahmad and E. V. McCollum. *Amer. J. Hyg.*, 1939, **29A**, 24).

Spectrographic examination of B₁₂ also showed the presence of phosphorus. Although nitrogen was found to be present, tests for sulfur were negative.

¹ We wish to thank Dr. Charles Rosenblum and Luise Anderson for this determination.

² Private communication from Dr. H. M. Walker, of Glaxo Laboratories, Ltd., discloses that Glaxo's factor also contains cobalt. E. L. Smith and L. F. J. Parker. *Proc. biochem. Soc.*, in press; E. L. Smith. *Nature, Lond.*, in press.

³ Personal communication; levels of 500 and 150 μg of cobaltous ion administered subcutaneously as acetate and chloride, respectively.

Microbiological assay of an aqueous solution of B₁₂ (74 $\mu\text{g}/0.5 \text{ ml}$) showed that autoclaving for 15 min at 121° C did not change the activity within the experimental error of $11.4 \times 10^6 \pm 0.6 \times 10^6 \text{ u}/\text{mg}$.

Vitamin B₁₂ in 0.015 N sodium hydroxide solution (0.1 $\mu\text{g}/\text{ml}$) was inactivated (microbiological assay) at room temperature as follows: 20% (0.67 hr), 45% (6 hrs), 90% (23 hrs), 95% (95 hrs); it was inactivated in 0.01 N hydrochloric acid solution (10 $\mu\text{g}/\text{ml}$) as follows: 18% (3 hrs), 75% (23 hrs), 89% (95 hrs).

The cobalt-complex nature of vitamin B₁₂ is an outstanding property.

EDWARD L. RICKES, NORMAN G. BRINK,
FRANK R. KONIUSZY, THOMAS R. WOOD,
and KARL FOLKERS

Research Laboratories,
Merck & Co., Inc., Rahway, New Jersey

A Laboratory Test for the Virus of Poliomyelitis

The need for a reliable test for the presence of the virus of poliomyelitis other than expensive monkey inoculations requires no emphasis.

Preliminary experiments indicate that such a test might be satisfactorily obtained through an interference phenomenon resulting in the protection of mice inoculated with material suspected of containing the virus of poliomyelitis against a subsequent inoculation with the Lansing strain of virus.

Virus is extracted and concentrated from the supposedly infected material in the usual manner (P. Lépine. *C. R. Soc. Biol., Paris*, 1939, **131**, 573). Five mice, 4-6 weeks of age, are injected intracerebrally with the extracted materials. Two days later the mice are given another intracerebral injection with active Lansing mouse-adapted virus. Five control mice are similarly treated, each receiving about 50 LD₅₀.

The answer is drawn from the results observed on the 10th-11th day, when 80% (4 out of 5), at least, of the controls should be paralyzed or dead, and at least 3 out of the 5 mice inoculated with the suspected material should show a significant protection. It should be mentioned that this significant difference between the two groups of animals may be the result either of permanent protection or of merely delayed onset of symptoms in the protected group.

Our experiments have included (a) cord material from monkeys inoculated with one standard laboratory strain and (b) fecal material collected from patients during the summer of 1946 and preserved in the frozen stage since that time.

Extracts from normal human feces, or cord material inactivated after heating at 80° C for 15 min, showed no protective action.

Attempts to obtain interference protection by other means of inoculation (oral, nasal, intraperitoneal, intracutaneous, etc.) or with unconcentrated material, or through a longer interval between inoculations, have so far failed.

It is hoped that these results will be found reproducible with strains of the virus of poliomyelitis other than the ones that have been tested.

PIERRE R. LÉPINE

Virus Division, Institute Pasteur, Paris

Facts and Theories About Sympathins

Marrazzi and Marrazzi (*Science*, November 28, 1947, pp. 520-521) have introduced in the discussion on sympathins a regrettable confusion between facts and theory.

The fact is that Cannon and Rosenblueth (*Amer. J. Physiol.*, 1933, 104, 557) observed more excitatory than inhibitory effects as compared with adrenaline during humoral transmission of certain sympathetic nerve stimulations. They never demonstrated that there was a purely excitatory and a purely inhibitory sympathin; and they have never been able to obtain more inhibitory than excitatory effects!

The elaborate theory built around this fact was not convincing, although it was accepted by a majority of Anglo-Saxon physiologists. We pointed out as early as 1933 that it was possible to give different interpretations of the phenomenon observed by Cannon and Rosenblueth, without postulating a combination of adrenaline with a hypothetical compound E or I in the cell. We suggested the following possibility: sympathin I = adrenaline; sympathin E = nor-adrenaline.

It is now evident from the work of von Euler (*Science*, April 23, p. 422), and that of many others mentioned by him, that one finds in suitable extracts of mammalian nerves and tissues a powerful amine which mimics more closely the actions of nor-adrenaline than those of adrenaline. We have confirmed von Euler's observations (*Arch. int. Physiol.*, 1947, 55, 73) with the important addition, however, that in certain tissues (human coronary arteries and nerves, for instance) the substance extracted has the properties of adrenaline and not of nor-adrenaline (or arterenol or dimethylated adrenaline) (Fig. 1). It must not be forgotten that the power of the adrenal medulla of the vertebrates to synthesize *l*-adrenaline is not unique. The parotid gland of tropical toads is rich in *l*-adrenaline (and recent observations from this laboratory show that it may contain other phenolic amines); certain cells in the abdominal ganglion of annelids synthesize an amine which has not been isolated in chemically pure state, but possesses in the utmost detail the properties of adrenaline (*J. F. Gaskell. J. gen. Physiol.*, 1919, 2, 73; *Z. M. Bacq. Biol. Rev.*, 1947, 22, 73). Von Euler has confirmed the conclusion of Leowi

(*Arch. ges. Physiol.*, 1936, 237, 504) that the sympathomimetic substance extracted from the frog's heart is adrenaline, the methylated amine.

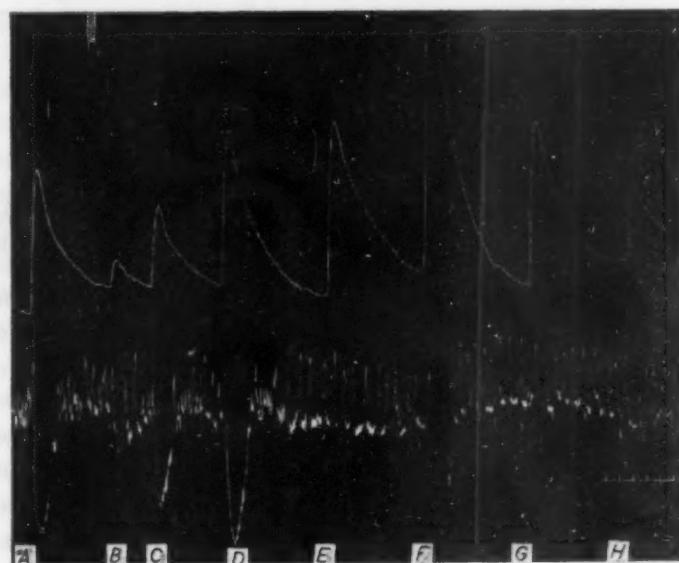


FIG. 1. Nonpregnant cat, Dial, curare. Above: nictitating membrane denervated 12 days previously. Below: uterus. Time in minutes. A, purified extract of 0.23 gm of human coronary nerves and arteries; B, *l*-adrenaline, 0.25 μ g; C, *l*-adrenaline, 0.5 μ g; D, *l*-adrenaline, 1 μ g; E, *dl*-nor-adrenaline, 1 μ g; F, G, H, purified extract of 0.75, 0.19, and 0.075 gm of horse spleen, respectively. This shows that the substance in the spleen acts very much like nor-adrenaline, but that the purified extract from human coronaries acts like adrenaline.

It seems to us that the best way to give a reasonable interpretation of these facts is to accept the idea that many tissues synthesize aminated derivatives of catechol, that the synthesis of adrenaline goes through arterenol—in other words, that the methylation of the nitrogen is the last step in this synthesis, according to Blaschko (*J. Physiol.*, 1942, 101, 337), and that this methylation does not occur in certain tissues.

The facts (not the theory) point to the existence of two highly active sympathomimetic substances: nor-adrenaline and adrenaline. Thus, von Euler's suggestion that our nomenclature be changed from sympathin E and sympathin I to sympathin N and sympathin A is logical; it would avoid further confusion until our knowledge of the relation between adrenaline and nor-adrenaline has sufficiently improved to remove all doubt.

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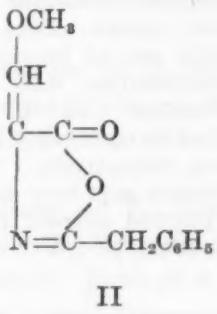
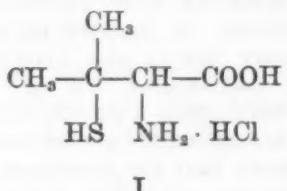
TECHNICAL PAPERS

A Synthesis of Benzylpenillic Acid¹

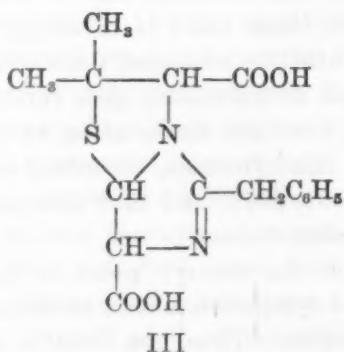
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Through a condensation of D-penicillamine hydrochloride (I) with 2-benzyl-4-methoxymethylene-5(4)-oxazolone (II), and subsequent treatment of the crude condensation product, a 19% yield of D-benzylpenillic acid (D-G-penillic acid) (III) has been obtained. The compound was identical with D-benzylpenillic acid prepared from benzylpeni-



II



illin by rearrangement in aqueous solution at pH 2. The structure of D-benzylpenillic acid has already been established by synthesis (1).

The condensation of D-penicillamine hydrochloride with 2-benzyl-4-methoxymethylene-5(4)-oxazolone was carried out for 15 min at 75° in pyridine containing 5% of triethylamine. The crude condensation product was freed of pyridine, triethylamine, and triethylamine hydrochloride and was dissolved in absolute methanol. When the methanol solution was allowed to stand at 22°, needles of D-benzylpenillic acid (micro m.p., 180–182°) formed slowly. The optical rotation of the synthetic D-benzylpenillic acid was $[\alpha]_D^{20}=+471^\circ$ (0.10% solution in methanol), while that of D-benzylpenillic acid prepared from

¹ This manuscript, submitted on March 29, 1946, for publication to follow the appearance of the projected monograph, *The chemistry of penicillin*, has been released for publication by the Editorial Committee of the monograph.

benzylpenicillin was $[\alpha]_D^{20}=+465^\circ$ (0.10% solution in methanol).

Starting with L-penicillamine hydrochloride, four diastereoisomers of benzylpenillic acid are possible, since there are three asymmetric carbon atoms in the benzylpenillic acid molecule. It is of considerable interest that the benzylpenillic acid isolated was the same isomer as that obtainable from natural benzylpenicillin.

When L-penicillamine hydrochloride was substituted for D-penicillamine hydrochloride in the procedure, L-benzylpenillic acid was obtained. This compound was identical with D-benzylpenillic acid in all respects, except for its opposite optical rotation, $[\alpha]_D^{20}=-476^\circ$ (0.09% in methanol). When DL-penicillamine hydrochloride was used, optically inactive benzylpenillic acid was obtained (micro m.p., 177–179°).

As already reported in detail in the penicillin monograph (3), the condensation of D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)-oxazolone in pyridine (containing no triethylamine) yields a small amount of antibiotic activity. A great deal of evidence indicates almost beyond doubt that this synthetical activity is due to benzylpenicillin.²

It has also been reported (3) that the concentration of acid in the pyridine solution greatly affects the formation of activity. On further investigation of this effect, we have found that an increase in the concentration of acid increases the rate of formation of activity but does not greatly affect the amount of activity formed. The addition of triethylamine to the pyridine solution, on the other hand, apparently interrupts the reaction at an intermediate stage. The crude product of such a condensation of D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)-oxazolone in pyridine solution containing 5% triethylamine possesses no antibiotic activity. Its ultraviolet absorption spectrum has a strong maximum at 3,200 Å ($E_M = 26,000-28,000$; calculated for a molecular weight of 334). The usual amount of activity can be produced, however, by heating this crude condensation product in pyridine solution containing pyridine hydrochloride (2).

In the course of fractionation studies on the crude product obtained by the condensation of penicillamine and the oxazolone in the presence of triethylamine, the formation of the D-benzylpenillic acid was encountered. The compound was obtained in a 5% yield from a rather concentrated solution of the crude condensation product in a mixture of hexane, chloroform, ethanol, and water which had been allowed to stand overnight at 22°. Sub-

² Since this paper was submitted for publication, benzylpenicillin has been isolated from this reaction mixture (V. du Vigneaud, F. H. Carpenter, R. W. Holley, A. H. Livermore, and J. R. Rachele. *Science*, 1946, **104**, 431).

ently it was found that a 10% yield could be obtained in 65% ethanol-35% water, and the yield was increased to 15% in absolute ethanol and 19% in absolute ethanol.

In a typical experiment, 60 mg of D-penicillamine hydrochloride and 66 mg of 2-benzyl-4-methoxymethylene-oxazolone were condensed in 11.4 cc of pyridine and 1 cc of triethylamine at 75° for 15 min. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 10 cc of chloroform. The chloroform solution was extracted with 10 cc of cold 2 M pH 1.6 phosphate buffer and with 10 cc of 1.5 M pH 5.2 phosphate buffer, and was dried over anhydrous sodium sulfate. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 2.0 cc of 95% ethanol. Crystals of benzylpenillic acid formed over a period of 24 hrs, and during this time the absorption at 3,200 Å gradually increased. After 44 hrs, 14.5 mg of D-benzylpenillic acid was obtained.

The identity of the compound was established by its melting point, mixed melting point with authentic D-benzylpenillic acid, ultraviolet absorption spectrum, optical rotation, and conversion to dimethyl D-benzylpenillate (1).

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Athropy of Ovaries Transplanted to the Spleen in Unilaterally Castrated Rats; Proliferative Changes Following Subsequent Removal of Intact Ovary¹

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It has been shown that the transplantation of an ovary to the spleen of a castrate female rat leads to the development of a luteoma in that ovary after a period of approximately 5 months. If the transplant is permitted to grow for 10 months or more, a granulosa cell tumor develops (1, 2). The major factor concerned is the hormonal imbalance that results from inactivation by the liver of the estrogenic and other hormones elaborated by the transplanted ovary. The histogenesis of the tumor

This investigation was supported in part by a research grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service. The authors are indebted to Richard Pencharz for his valuable collaboration.

has been described (2). The important changes are the continuous formation of new primordial follicles, transformation of the follicles into corpora lutea, and growth of the corpora lutea with the formation of a large encapsulated luteoma in which granulosa cell nests arise (2). The appearance of these tumors after intrasplenic ovarian transplants in mice, guinea pigs, and rabbits has been described (3, 5, 6, 7). Confirmation of the growth in rats of the ovarian transplant (4) and formation of the tumors (7) has been published.

During the original experiments it had been found that if adhesions formed between the spleen and the systemic circulation so that the ovarian hormones bypassed the liver, the growth of the transplant was greatly retarded or would not take place. It was also noted that a pellet of estrogen placed in the subcutaneous tissue had the same effect on the transplanted ovary.

In the present experiments rats of the Long-Evans strain were used. In the first group there were 3 immature and 3 mature females. In the case of each animal one ovary was removed, cleaned of adherent structures, and placed in a pocket under the capsule of the spleen. The other ovary was not disturbed. One animal was sacrificed at each of the following days after transplantation: 24, 55, 90, 130, 150, and 246. In all animals the ovary *in situ* had undergone the usual compensatory hypertrophy that takes place after unilateral ovariectomy. The gross and histologic changes were the same in the immature and mature animals. The transplant in the spleen was much smaller than the original ovary; its position was indicated by the scar on the capsule of the spleen. Histologic preparations were made in the customary manner, and serial sections were studied. The changes were uniform except that in the youngest transplant, after 24 days, there were many small, active follicles with intact ova. No corpora lutea were present. The interstitial tissue was distorted by fibrous tissue. In all of the remaining animals the histologic structure was characterized by extremely few follicles that were relatively small in size. If ova remained, they appeared degenerated. There were no newly formed corpora lutea, and those that had been present in the mature ovaries had disappeared. The interstitial tissue was increased. In the animal examined after 246 days the degree of atrophy was severe; only two minute follicles were noted in a dense, fibrous interstitial tissue.

The second series of 4 immature animals was prepared by transplanting the left ovary into the spleen. After intervals of 63, 91, 91, and 91 days the hypertrophied right ovary was removed. After additional periods of 69, 97, 122, and 143 days the animals were sacrificed. Gross examination showed enlargement of the transplant. In the 3 oldest it was approximately 1 cm in diameter. Each uterus was atrophic, and the vaginal smears showed mainly leucocytes. Serial sections of the transplants were prepared. In the 69-day-old specimen the ovary was replaced by congeries of closely packed, large corpora lutea. Each corpus was quite distinctly outlined, and usually contained a small, central fibrous core. Scattered throughout were a few developing follicles. After 97 and 122 days the pattern was similar except that a rare fol-

licle was cystic. After 143 days there was some loss of demarcation of the corpora lutea so that fusion of the corpora was suggested. Rarely was a fibrous core visible in these corpora. Primordial follicles were not evident. The picture suggested that the corpora were coalescing toward the formation of the luteoma.

Summary. An ovary placed in the spleen of an animal that has one normal ovary undergoes atrophy that starts as early as 24 days after transplantation. Severe atrophy is reached before 55 days, and subsequent changes are minor. The degree of atrophy is almost as intense as that noted in the hypophysectomized castrate animal with an ovarian transplant in the spleen (2). The transplanted ovary shows no evidence of any type of response to the hormonal influence that has stimulated the ovary that remained *in situ*. Removal of the normal ovary introduces the hormonal imbalance that was present in the original tumor animals. The atrophic ovarian parenchyma in the spleen in these, as well as in the hypophysectomized animals previously reported, retained its growth potentials and responded to the hormonal forces by assuming the changes that characterized the development of the luteoma. The action of the atrophic ovarian parenchyma is reminiscent of malignant cell deposits that may remain dormant or quiescent for long periods and then, for some unknown reason, suddenly undergo growth.

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Effect of Ryanodine on the Oxygen Consumption of *Periplaneta americana*

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Extracts of the plant *Ryania* have been found to possess insecticidal properties and are being investigated for physiological effects. Injections of the material produce paralysis and death in insects. The paralysis is of such nature that no responses can be obtained to mechanical or electrical stimuli, yet the oxygen consumption rises very noticeably. The oxygen uptake of the cockroach, *Periplaneta americana*, has been studied in the Fenn differential manometer before and after the injection of Ryanodine (concentrate of active principles of Ryanex ethanol solution, furnished by Merck & Co., Inc.).

Adult roaches of both sexes were used. Those used for controls were immobilized by confinement in a jacket

consisting of a section of braided wire cable shield of suitable size. These jackets were flexible enough to permit insertion of the animals without harm but

TABLE 1

O_2 UPTAKE AND PERCENTAGE OF CHANGE BEFORE AND FOR PERIOD OF 30 MIN-2½ HRS AFTER THE INJECTION OF RYANODINE EXTRACT OR RINGER SOLUTION

Ryanodine		Change (%)	Saline solution	
mm ³ /O ₂ /gm/min	Before injection		mm ³ /O ₂ /gm/min	Before injection
5.14	16.41	203.3	5.97	6.45
3.91	12.09	209.2	4.84	6.91
1.96	7.89	302.6	4.34	6.72
6.71	20.34	203.1	2.27	5.35
6.04	18.12	200.0	6.34	10.34
9.25	14.52	57.0	6.06	15.55
6.02	17.76	195.0	10.12	11.87
5.43	15.20	179.9	7.12	8.56
5.00	14.69	193.8	5.90	5.26
7.25	16.52	127.9	9.23	9.14
9.14	13.75	50.4		
1.06	8.23	676.4		
4.61	14.15	206.9		
6.87	12.80	86.3		
5.16	13.12	154.3		
2.51	14.98	496.8		
5.88	17.00	189.1		
1.98	10.17	413.6		
2.94	8.57	191.5		
2.23	4.49	101.3		
Means	4.97	13.54	220.0	6.22
				8.62

enough to prevent movement; they were also placed in jackets of suitable size. These jackets were flexible enough not to interfere with the movement of gases. Adult roaches were weighed, slipped into the jackets, and placed in the manometer vessels, after which the O_2 uptake was measured for 2-2½ hrs at 25° C. They were then moved, and some were injected intrasternally with 0.01 ml of 0.01% Ryanodine, in an insect Ringer solution. The roaches were then placed back in the jackets and the O_2 uptake was measured again for 2-2½ hrs at 25° C.

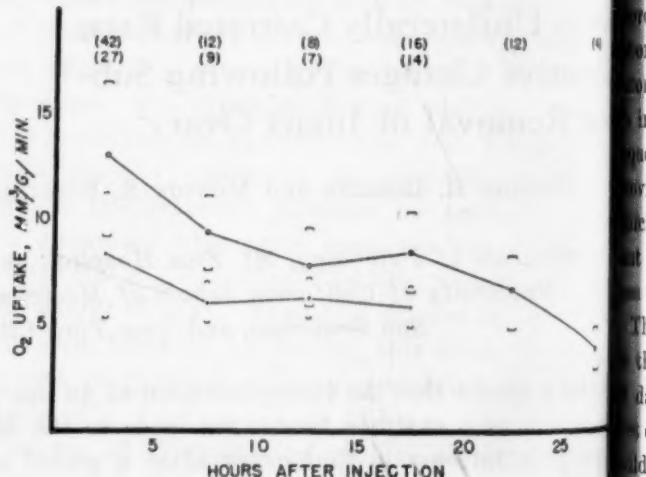


FIG. 1. Decrease in O_2 uptake, over a 30-hr period, in Ryanodine- and saline-injected roaches. Experimental series, ○; controls, ●. The points represent the mean values for 5-hr periods, and the figures in parentheses above give the number of readings represented by each point. The brackets above and below the points represent the standard deviation of the mean value for each point.

was measured for 2-2½ hrs at 25° C. They were then moved, and some were injected intrasternally with 0.01 ml of 0.01% Ryanodine, in an insect Ringer solution. The roaches were then placed back in the jackets and the O_2 uptake was measured again for 2-2½ hrs at 25° C.

ers were injected with an equal amount of the saline solution alone. They were then returned to the manometers, and the O_2 uptake was measured for another period of 2-2½ hrs. Because of the time consumed in injecting and setting up and equilibrating the vessels, measurements began about 30 min after the treatment. This delay prevents measurement of the initial rise. Subsequently the O_2 consumption of the animals was measured after longer periods, up to 80 hrs.

definite increase in O_2 consumption was recorded injection of the Ryanodine. Table 1 shows that the rate of $4.97 \text{ mm}^3/O_2/\text{gm}/\text{min}$ for normal animals increased to $13.54 \text{ mm}^3/O_2/\text{gm}/\text{min}$, or 222%, while for animals injected with saline solution the rate changed only from 6.22 to $8.62 \text{ mm}^3/O_2/\text{gm}/\text{min}$, or

high O_2 consumption was maintained for a considerable time, as shown in Fig. 1. By the end of the 24 hrs it had dropped to the normal level, and later, between 30 and 40 hrs, it decreased to a constant level $mm^3/O_2/gm/min.$ Measurements made between 40 and 80 hrs showed no further change, and this level probably indicates the onset of death. Because of the total lack of movement after the Ryanodine injections, the time of death was otherwise indeterminate.

New Method of Reporting Data on Production and Lactation in Mouse¹

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studying the nutritive requirements of the mouse for production and lactation, it was the practice in this laboratory, in common with that observed in other laboratories, to consider all survival failures as being due to inability of the mothers to nurse the young, and, consequently, as indicative of the inadequacy of the diet to produce normal lactation. However, the persistency with which newborn mice died during the first 4 days of life cast suspicion on the reproduction rather than the lactation performance.

The idea that the maternal diet exerts a great influence on the health of the newborn is not new. In fact, a host of data has been accumulated concerning the role of the diet of the mother with regard to the welfare of the unborn infant. For example, Burke, *et al.* (1) have observed a statistically significant relationship between the quality of the diet consumed by the mother during pregnancy and the health of the infant at birth. Every stillborn infant, every infant dying within a few days after birth, the majority of babies with marked congenital defects, all premature and "functionally immature" infants

This investigation was aided by grants from the John and R. Markle Foundation and the National Vitamin Foundation, Inc. A preliminary report of the present paper has already appeared (American Chemical Society Abstracts, New Meeting, September 1947).

were born to mothers whose diets were inadequate. At the prenatal clinic in Toronto, Ebbs and Moyle (2) found that the number of miscarriages, stillbirths, premature births, and infant deaths was greatest in the case of mothers receiving a poor diet. Lastly, in spite of inevitable suffering and hardships endured by women in England during the war years, babies born in that country have been heavier and longer in their first year of life. According to Garry and Wood (4), nutrition was the only factor which improved in England during the recent war.

Yet, various investigators who have studied the effect of purified diets on experimental animals are of the opinion that the problem of reproduction has been solved, whereas that of lactation still awaits solution. Spitzer

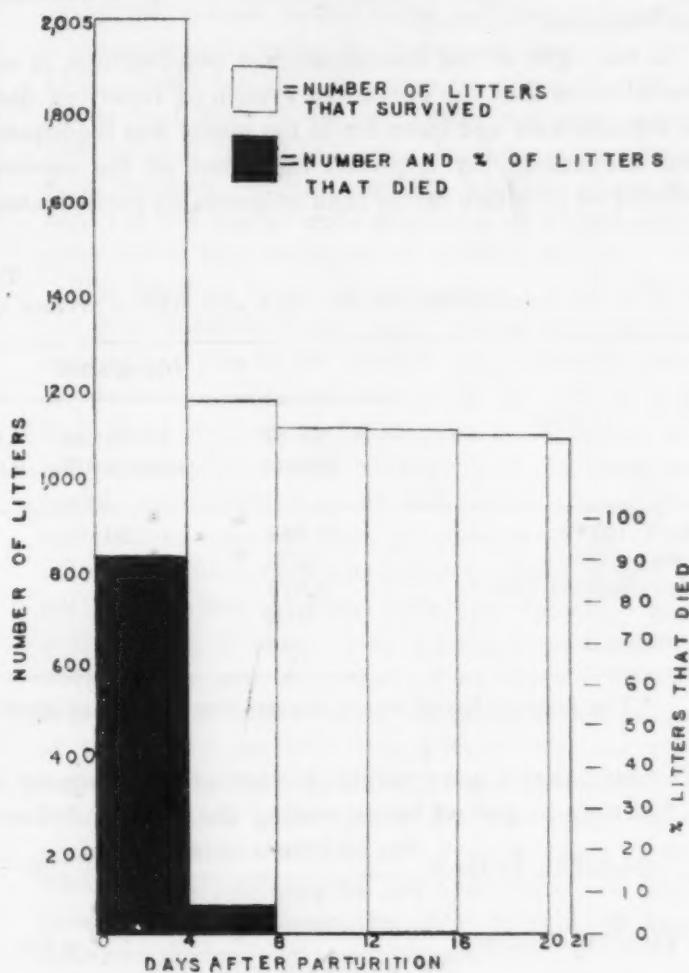


FIG. 1

and Phillips (6) state: "Young that were born alive appeared normal at birth, but did not live more than 1 or 2 days after parturition. Although the young attempted to nurse, no milk could be found in their stomachs. When this condition was observed in these experiments it was considered to be due to lactation failure." A similar conclusion was drawn by Fenton and Cowgill (3): "Reproduction and lactation have been studied in highly in-bred strains of mice fed purified diets. . . . The problem appears to be one of lactation."

It is our contention, supported by observations on 2,005 litters, that the problem is primarily one of reproduction and not of lactation. If a diet is qualitatively adequate for reproduction, it will also be, according to our findings, adequate for lactation. However, the converse does not necessarily follow. Fig. 1 shows that, of a total of 900

litter deaths, 91% succumbed during the first 4 days of life and 9% during the remaining 17 days of the lactation period. The deaths which occurred during the first 4 days were a consequence of the poor maternal diet, for in all instances the young were puny, undersized, and weak. Thus, whereas the first month is critical for the human infant, in the case of the mouse the first 4 days are decisive, and the outcome depends on the quality of the maternal diet. This is supported by observations that have been made in this laboratory on 127 mothers whose litters succumbed during the first 4 days of life. Fifty-seven of these were able to rear foster litters born to females kept on stock or good experimental diets. The conclusion seems to be justified, therefore, that the deaths of the natural litters were not due to a poor lactation performance.

In the light of the aforementioned observations, it appeared to us that the prevailing system of reporting data on reproduction and lactation in the mouse was inadequate and misleading, for it placed the brunt of the survival failures on lactation rather than on gestation performance.

In Table 1, the results of this investigation are recorded according to the old and new systems of reporting. The experiments were carried out on mice belonging to the following strains: Rockland albino, C 57 Black, Webster, and our own albino strain which we have inbreeding for the last 12 years.

By way of illustration, we have included in the table data obtained with two of our good rations, diets R-5a and RB²S-20. These are modifications of our basal diet R-5a, which consisted of casein (Labco or GBI) (30%), sucrose (48%), salt mixture (5%), lard (5%), hydrogenated vegetable oil (10%), Ruffex (2%), and contained the following supplements per kilo of diet: thiamine, 1 mg; riboflavin, 20 mg; pyridoxin, 20 mg; calcium pantothenate, 40 mg; α -tocopherol, 20 mg; vitamin A concentrate, 67.5 mg (67,500 I.U.); vitamin D (Dried 5,000 units; and choline chloride, 500 mg. Diet RL-5a consisted of diet R-5a, to which was added an aqueous extract of liver (3 gm/100 gm of diet). Diet RB²S-20 consisted of diet R-5a, to which was added 200 γ of biotin and 100 mg of pteroylglutamic acid per kilo.

TABLE 1
COMPARISON OF THE OLD AND NEW SYSTEMS OF REPORTING DATA ON REPRODUCTION AND LACTATION

Diet	Old system				New system			
	No. of litters	Lactation success (%)	Gestation success (%)	Gestation index	Viability index	Over-all reproduction index	Lactation index	
Stock (3)*	250	90	96	96	92	88	95	
Basal (3)*	239	42	62	62	49	30	86	
Experimental (36)*	1,376	71	80	80	72	58	91	
RL-5	104	70	84	84	72	60	95	
RB ² S-20	36	80	97	97	80	78	100	

* The figure in parentheses denotes the number of diets used.

We found that a more reliable picture of the adequacy of a diet may be gained by expressing the data as follows:

$$\text{Gestation Index} = \frac{\text{No. of litters born alive}}{\text{No. of pregnant animals}} \times 100$$

$$\text{Viability Index} = \frac{\text{No. of litters alive on 4th day}}{\text{No. of litters born alive}} \times 100$$

$$\text{Over-all Reproduction Index} = \frac{\text{No. of litters alive on 4th day}}{\text{No. of pregnant animals}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of litters weaned}}{\text{No. of litters alive on 4th day}} \times 100$$

The 'Over-all Reproduction Index' has been added to the other three indices, which were discussed in the preliminary report of this investigation. Addition of this index is based on our findings that if a litter survives the first 4 days of life, its chance of reaching the end of the suckling period is close to 90%. Now then, if we are correct in assuming that the nutritional condition of the mother before and during gestation is decisive with regard to the ability of the young to survive, of the four indices, the 'Over-all Reproduction Index' would seem to be the most important for evaluating the adequacy of a diet for reproduction and lactation in the mouse.

It is interesting to note that, according to the old system of recording data, the lack of viability was considered a lactation failure. The new system indicates on the other hand, that whereas a purified diet may be inadequate for the production of viable young, it can be adequate for normal lactation performance. In fact, as shown in the table, the lactation index is high, even for the basal diets.

On the evidence of the results obtained, we may therefore conclude that in the case of the mouse, as in that of the rat (5), the nutritional requirements for a normal gestation leading to the birth of viable young are more stringent than those for lactation.

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IN THE LABORATORY

A Staining Procedure for the Study of Insect Musculature¹

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Microdissection and study of insect musculature is frequently a slow and tedious task. When adult insects are killed with no particular effort to assure differentiation and recognition of the muscles, the difficulties of locating their exact origins and insertions are considerably increased. It is, however, possible to obtain good preparations of adult insect muscles in many cases by the use of special killing and fixing agents. Chloral hydrate and several fixing fluids containing formaldehyde can be used to distinguish the muscles from other internal tissues.

With soft-bodied larval forms of insects such as the Diptera, which frequently have a large amount of adipose tissue running through the body cavity, the problem of differentiation becomes more acute. When killed and fixed, the muscles of such larvae are often the same milky white color as the fat body and other internal tissues, and the origins and insertions of the separate muscles are confused. In other cases the muscles remain completely transparent and unrecognizable.

In the hope of finding some method of obtaining good *in toto* muscle differentiation, a series of killing and staining procedures was undertaken. Reared, 4-day-old housefly larvae were used in these procedures. One of the attempts not only gave remarkable differentiation of the muscles, but made it possible to see almost every separate muscle quite clearly when viewed with a binocular microscope, directly through the body cuticle, thus eliminating the need for dissection. In addition, due to the peculiar optical properties which this technique brings about, it is possible to see first the outermost body muscles, then the intermediate muscles, and finally the innermost muscle bundles by rotating the specimens in the light. As any one layer of muscles is visible in a given position in the light, the other muscles are almost transparent.

The steps in this procedure are: (1) Bouin's fluid (30° C), 8-10 hrs; (2) 50% ethyl alcohol, 10 min; (3) 70% ethyl alcohol, 1 hr; (4) 95% ethyl alcohol, 10 min; (5) 0.5% eosin alcohol, 6-8 hrs; (6) return to 95% alcohol and add oil of wintergreen dropwise to larvae in 95% alcohol at hourly intervals over a period of 4-5 days; and (7) transfer larvae to oil of wintergreen.

The Bouin's fluid should be prepared with formalin solution which has been neutralized with magnesium sulfate. The larvae die slowly when dropped into Bouin's fluid, permitting the fixative to penetrate the body tissues. Most of the larvae are killed within an hour or two, but

¹Contribution No. 285, Department of Entomology, University of Illinois, Urbana.

occasional larvae may stay alive in this fluid for 4 hrs. After being removed from the fixative and washed in 50% ethyl alcohol, the larvae are turgid but exceedingly soft and pliable. The outer cuticle has a glistening appearance and is entirely transparent, as are the muscles. The silvery luster of the main longitudinal tracheal trunks and their branches are distinctly visible through both the cuticle and body muscles. Also visible are the fat body, the ventral nerve branches, the alimentary canal, the cephalopharyngeal mouth hooks, and other internal organs.

As indicated above, the larvae are then transferred to 70% alcohol for 1 hr, but may be stored in 70% alcohol for several days or longer. They are then removed to 95% alcohol for 10 min prior to being placed in a 0.5% eosin solution (wt/vol) in 95% alcohol, where they are kept for 6-8 hrs, or until they take on a light pink color, after which they are returned to 95% alcohol.

The addition of oil of wintergreen, which will replace the 95% alcohol, is the most critical step in this procedure. If this is not carried out gradually, either the larvae will collapse and shrivel or the cuticle may split and the body organs be displaced. A dozen or more larvae are placed in a stendor dish, or other suitable vessel, with about 8 cc of 95% ethyl alcohol, which is sufficient to immerse them completely. Four to 6 drops of oil of wintergreen are added to the dish and swirled until uniformly miscible with the alcohol. This procedure is repeated at about hourly intervals during the working day over a period of 4-5 days. The stendor dish should be kept covered to prevent excess evaporation of the alcohol, as such evaporation will also cause collapse of the larvae. Occasional examination of the larvae should be made with a binocular microscope during the period of addition of the oil of wintergreen in order to detect possible collapse of the specimens. If the larvae show signs of flattening, the oil of wintergreen should be added at intervals of several hours.

With the addition of oil of wintergreen, the larvae become opaque as the muscles become visible. The larvae retain their pink color for about 4 days and then turn orange red. At this point the remaining alcohol may be evaporated from the solution by removing the lid of the stendor dish for several hours. The larvae are then transferred to oil of wintergreen.

When a spot of light is directed into any portion of a larva as it is studied beneath a binocular microscope, the larva takes on a yellow-green color. The green-tinted cuticle is transparent with distinctly visible opaque muscles beneath. The individual muscle fibers of each muscle band can be seen readily, and their exact insertions on the cuticle are visible. As a larva is moved in the light, the different muscles of the various muscle layers show up distinctly, and it is possible to obtain an accurate knowledge of the relationship of almost all somatic muscles in this way.

This technique has given good results with other diplopterous larvae such as the rat-tailed maggot (*Eristalis* sp.) and has rendered visible the muscles of the head of mosquito larvae through the head capsule. It has also proved applicable for the study of the relationship of some of the thoracic muscles of an adult homopteran insect (*Ceresa bubalus*) and was particularly useful for delineating the muscles of the small labium. It is possible that the staining and optical properties of this technique, with various modifications, may be useful for the study of the musculature of other insects and soft-bodied invertebrates.

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A Practical Method for the Illumination of Biological Material With Ultraviolet Rays

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The study of self-fluorescence in tissues and vital staining with fluorescent dyes in the living body requires a source of ultraviolet light that is intense and at the same time constant. Until recently we had been unable to find a source of ultraviolet light embodying all these features. The arc light was usually used when a source of ultraviolet light was needed in a laboratory. However, it always had three major drawbacks: (1) it gave off an intense amount of heat which made the operator rather uncomfortable; (2) the automatic carbon feeder never worked properly; and (3) the carbons never lasted more than 30-45 min, usually extinguishing themselves just when something of interest was visualized. Then, also, there had to be a short delay for the lamp housing to cool off so that the operator could change the carbons. This caused the investigator who was studying the movement of fluorescent solutions in the living body to miss a part of the action taking place during this interval. After much trial and error it was found that certain microscope lamps available on the market could be easily converted into a very efficient source of constant and intense ultraviolet light.

For general all-round efficiency, the Bausch & Lomb spherical lamp housing was found to be the best in the low-priced field. The B & L research lamp housing can be used with one minor change. The Spencer lamp housing (#370-A) is also readily converted, but in our experience it was found to have some disadvantages.

In converting the B & L spherical lamp housing, we removed the bayonet type socket and substituted a porcelain-shelled admedium socket (GE #3280). To hold this socket in place and to enable regulation of its vertical movements, a special holder was constructed along the lines of the original holder for the bayonet type socket. This holder was made in the form of a ring to encircle

the admedium socket, a lip being made on each end so that the ring could be tightened around the socket. On opposite sides of the ring were brazed two slotted upright pieces which would fit under the thumb screws of the lamp housing provided for this purpose. These thumb screws

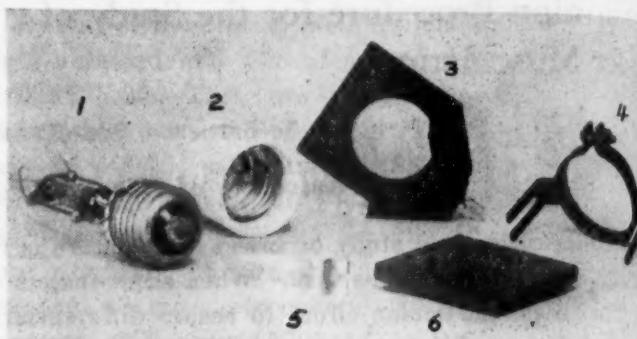


FIG. 1. Accessories: 1, AH-4 mercury vapor lamp; 2, admedium lamp socket; 3, filter holder; 4, socket holder; 5, slotted holder for filter holder; 6, ultraviolet filter.

hold the socket stationary after the vertical centering of the lamp is accomplished. To complete the lamp housing we used a B & L filter holder, which was placed in front of the iris diaphragm. To hold it in place a piece of metal was slotted on both ends and bent in such a manner that the opening of the filter holder was centered with respect to the condenser. One slotted end of the metal strip is slipped under the thumb screw holding the iris diaphragm in place, while the opposite end is used to hold the filter holder in place by its thumb screw. Fig. 1 shows the individual parts of the ultraviolet lamp and in Fig. 2 the completely assembled lamp is seen.

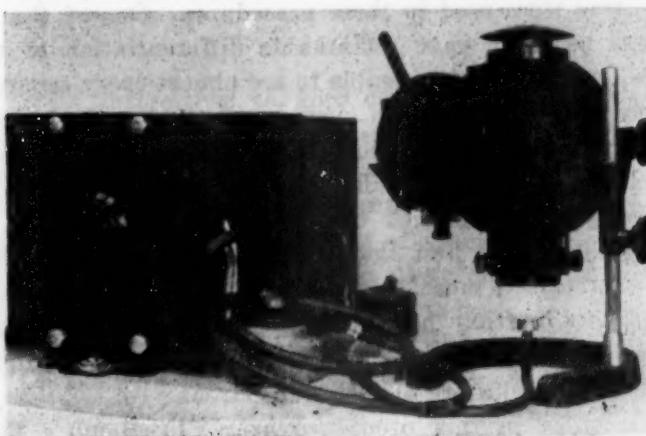


FIG. 2. Assembled ultraviolet lamp including transformer and lamp housing.

The constant and intense source of ultraviolet light we use is the General Electric AH-4 100-watt mercury vapor lamp. To operate this lamp a General Electric Autotransformer No. 59G22 is absolutely necessary.¹

For filtering out the visible light a number of glass filters are available. We have found that those most suited for ultraviolet light are the Corning glass filters

¹ Our AH-4 mercury vapor lamp, porcelain admedium socket, and autotransformer were obtained from G. W. Gates & Co., Franklin Square, Long Island, New York.

numbered 5860, 5840, 5874, 9863.² When the lamp is used for fluorescent microscopy, it is necessary to place in the ocular(s) a 20-mm circular Corning glass absorption filter (No. 3060) to prevent injury to the eye from ultraviolet rays passing through the objective.³

Where a greater amount of ultraviolet light is required, we had the surface of the B & L spherical lamp housing reflector aluminized by the Alzak process and then substituted two spherical quartz condensers in place of those originally supplied. This, however, is not necessarily required for all work.

For greater efficiency in fluorescent microscopy, the microscope condenser was replaced by one made of quartz. An aluminum-surfaced disc placed over the microscope mirror or resurfacing of the mirror with aluminum by the Alzak process is also required for fluorescent microscopy.

For ultraviolet rays below 3,650 Å the outer glass envelope was removed from the lamp.

The above method is the one used by us in work in this field which has been reported recently (1).

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A New Tool for Infrared Studies

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During the last 10 years the writer has studied in infrared light the optical properties of many minerals which are opaque in visible light. The wave length used was 9,000 Å (1).

By substituting a photoelectric ocular, sensitive to infrared light, for the ordinary eyepiece of a polarizing microscope or other optical apparatus used in mineralogical studies, it is possible to study the optical properties of many opaque minerals, using the same techniques as for nonopaque minerals. The photoelectric current produced by the optical phenomena studied is amplified and measured by the use of a sensitive galvanometer.

This method gives a high degree of precision. For example, during the measurement of refractive index, the maximum deflection of the galvanometer can be accurately noted. When using index oils, different persons estimate the point at which the index of the oil and the mineral match with slightly different degrees of precision.

During the last war, the American and German armies improved and used the sniperscope and snooperscope for night fighting.

Corning glass filter No. 9863 passes ultraviolet light down to 2,500 Å but passes a trace of red and violet.

These filters were obtained from the Corning Glass Works, Corning, New York.

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The field one wishes to observe is illuminated by a beam of infrared invisible light. By the use of a telescope containing an electronic infrared image tube, the observation of the field is about as simple as it is in daylight.

The invisible image produced by the lens of the telescope is projected on the front half-transparent cathode of the image tube. Inside the tube, the electrons pulled out of the cathode are focused by a set of electronic lenses and hit the rear fluorescent screen of the tube, producing a visible image of the observed field. The resolution is very high—nearly the same as for a television tube.

With the help of the Electronics, Physics, and Geology Departments of Washington University, the writer obtained an image tube and adapted it to a polarizing microscope (Fig. 1).

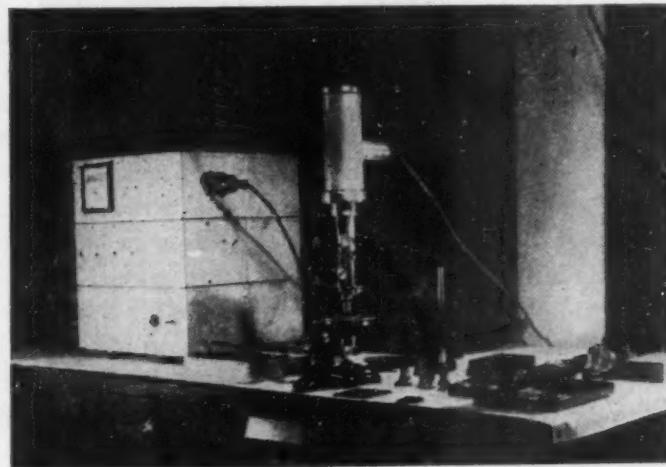


FIG. 1

The results are extraordinary. It is possible to study in infrared light a section of molybdenite (MoS_2) about $\frac{1}{2}$ mm thick, as are ordinary transparent minerals. In visible light, sections of molybdenite, $1/100$ mm thick are opaque. Stibnite (Sb_2S_3) is transparent in sections 3 or 4 mm thick. Natural pure antimony can be studied in sections up to about 1 mm thick.

Interference figures can be observed as in visible light.

The sensitivity of the image tube is great. The manipulation of this new infrared microscope is exactly the same as for an ordinary polarizing microscope. The only difference is the green color of the image, which is produced by the fluorescent screen.

The image tube can be adapted to the ocular of a refractometer, goniometer, and other apparatus used in mineralogy and petrology. Thin sections used in paleontology and paleobotany are particularly interesting to study in infrared light. Internal structures of Foraminifera and other small animals can be discerned. With the infrared image tube, the long, tedious study by photography with the use of special plates is eliminated.

Observation with infrared light, with this new technique, should be very useful for medicine, biology, chemistry, and allied sciences.

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Book Reviews

Preparation and characteristics of solid luminescent materials. Gorton R. Fonda and Frederick Seitz. (Eds.) New York: John Wiley; London: Chapman & Hall, 1948. Pp. xv + 459. (Illustrated.) \$5.00.

This volume consists of papers and discussion presented at the conference on luminescence held at Cornell University in October 1946, under the sponsorship of the Division of Electron and Ion Optics of the American Physical Society. As might be expected, it deals with a wide variety of topics and presents points of view which are sometimes widely divergent; nevertheless, because of the careful organization of the conference and of the book, it gives a well-balanced picture of this rapidly developing field. It opens with four broadly general chapters on the preparation, structure, and properties of phosphors. The next two chapters, also general in character, present theoretical analysis of a number of features in their behavior. The introductory discussion of the subject concludes with chapters on infrared phosphors and on excitation and emission processes in phosphors. The remaining chapters deal in a more specialized way with a wide variety of subjects: changes in fluorescence characteristics with composition, temperature, and intensity of excitation; energy storage in phosphors; the growth and decay of phosphorescence; factors affecting absorption and emission spectra; photochemical effects in luminescent materials; correlations between the structure and luminescence of materials; and so on. The book concludes with a summary of the final discussion of the conference, indicating what needs to be done, experimentally and theoretically, to gain a better understanding of luminescence.

The book gives, to a surprising degree, the atmosphere of a conference: the sometimes bewildering variety of subjects; the conflicts in point of view and sometimes even in experimental results; the abundance of unanswered questions; and the stimulation of surveying a subject in the course of rapid development. A beginner in the field might wish for a more carefully unified and polished account of the subject, but such a book cannot yet be written without danger of serious oversimplification of the subject. At any rate, he will find here references to the older, as well as to recent, literature, a reasonably complete summary of established facts, a good indication of the directions of present-day development, and a wealth of suggestions for future work. Indeed, this book may be as valuable for the questions it raises as for the answers it provides.

In view of the large number of items of information on various materials scattered through the book, it is unfortunate that no index is provided. In all other respects it can serve as a model for similar volumes dealing with other subjects undergoing such rapid development that a definitive text cannot yet be written.

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